INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01780

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 63/00; A61K 48/00; C07H 21/02, 21/04
US CL : 424/93.2; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.2; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, APS, DIALOG
search terms: sapa, sap(w)a, campylobacter fetus?, mutant?, heterologous, foreign protein?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DWORKIN, J. et al. Generation of Campylobacter fetus S-layer protein diversity utilizes a single promoter on an invertible DNA segment. Molecular Microbiology, 1996, Vol. 19 (6), pages 1241-53, see abstract.	1-9
Y,P	FUJITA, MASAKI et al. A deletion in the sapA homolog cluster is responsible for the loss of the S-layer in Campylobacter fetus strain TK. Archive of Microbiology, 1997, Vol. 167, No. 4, pages 196-201, see abstract.	1-9
Y	DWORKIN, J. et al. Segmental conservation of sapA sequences in type B Campylobacter fetus cells. Journal of Biological Chemistry. 1995, Vol. 270, No. 25, pages 15093-15101, see abstract.	1-9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 31 MARCH 1998	Date of mailing of the international search report 28 MAY 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GINNY PORTNER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01780

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DWORKIN, J. et al. A lipopolysaccharide-binding Domain of the Campylobacter Fetus S-layer protein resides within the conserved N-terminal of a family of silent and divergent homologs. Journal of Bacteriology, April 1995, Vol. 177, No. 7, pages 1734-1741, see abstract.	1-9
Y	FUJITA, M. et al. Localization of the sapa gene on a physical map of Campylobacter fetus chromosomal DNA. Archives of Microbiology, 1994, Vol. 62, No. 6, pages 375-380, see abstract.	1-9
Y	TUMMURU, M.K.R. et al. Rearrangement of sapA homologs with conserved and variable regions in Cappylobacter fetus. Proceedings of the National Academy of Science, U.S.A., August 1993, Vol. 90, No. 15, pages 7265-7269, see abstract.	1-9
A	TUMMURU, M.K.R. et al. Characterization of the Campylobacter fetus sapA promoter: evidence that the sapA promoter is deleted in spontaneous mutant strains. Journal of Bacteriology, September 1992, Vol. 174, No. 18, pages 5916-5922, see abstract.	1-9
Y	YANG, LY et al. Reattachment of surface array proteins to campylobacter fetus cells. Journal of Bacteriology, February 1992, Vol. 174, No. 4, pages 1258-1267, see entire document.	1-9
X	BLASER, M.J. et al. High-frequencey S-layer protein variation in Campylobacter fetus revealed by sapA mutagenesis. Molecular Microbiology, 1994, Vol. 14, No. 3, pages 453-462, see figure 1 and whole reference.	1-4, 6-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01780

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions r groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-9, drawn to a mutant Campylobacter fetus strain and a DNA cassette encoding a heterologous protein, wherein the C. fetus strain is a sapA homolog.
Group II, claim(s) 10-13, drawn to Campylobacter fetus mutant strains wherein a recA mutation is present together with a heterologous antigen or a sapA gene mutation.
Group III, claim(s) 14-17, drawn to any strain of bacteria which has been modified to express SapCDEF genes.
The inventions listed as Groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Each group contains mutant strains which evidence specific structural differences in the genes that are altered or added to the mutant strain. Therefore, each group represents mutant strains which have differing special technical features which have differing structures, functions and effects on the over all end product.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference D5979PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US98/01780	International filing date *(day/month/year)* 30 JANUARY 1998	Priority date *(day/month/year)* 31 JANUARY 1997
International Patent Classification (IPC) or national classification and IPC IPC(6): A01N 63/00; A61K 48/00; C07H 21/02, 21/04 and US Cl.: 424/93.2; 536/23.1		
Applicant VANDERBILT UNIVERSITY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [X] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [X] Lack of unity of invention

   V [ ] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23 SEPTEMBER 1998	26 JANUARY 1999
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GINNY PORTNER Telephone No. (703) 308-0196

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US98/01780

I. Basis of the report

1. This report has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments)*:

☐ the international application as originally filed.

☒ the description, pages 1-6, 8-29 , as originally filed.
pages NONE , filed with the demand.
pages 7 , filed with the letter of 08 January 1999.
pages _____ , filed with the letter of _____.

☒ the claims, Nos. 10-17 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. 1-9 , filed with the letter of 08 January 1999.
Nos. _____ , filed with the letter of _____.

☒ the drawings, sheets/~~fig~~ 1-7 , as originally filed.
sheets/~~fig~~ NONE , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☒ the description, pages none.

☒ the claims, Nos. none.

☒ the drawings, sheets/~~fig~~ none.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

[X] the entire international application.

[ ] claims Nos. _

because:

[ ] the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

[ ] the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

[ ] the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

[X] no international search report has been established for said claims Nos. 10-17.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- [X] restricted the claims.
- [ ] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. [ ] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- [ ] complied with.
- [X] not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-9, drawn to a mutant Campylobacter fetus strain and a DNA cassette encoding a heterologous protein, wherein the C. fetus strain is a sapA homolog.
Group II, claim(s) 10-13, drawn to Campylobacter fetus mutant strains wherein a recA mutation is present together with a heterologous antigen or a sapA gene mutation.
Group III, claim(s) 14-17, drawn to any strain of bacteria which has been modified to express SapCDEF genes.
The inventions listed as Groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Each group contains mutant strains which evidence specific structural differences in the genes that are altered or added to the mutant strain. Therefore, each group represents mutant strains which have differing special technical features which have differing structures, functions and effects on the over all end product.

In response to the arguments presented in the "Response to Written Opinion" submitted January 08, 1999, the examiner has considered these arguments and the amendment to the claims and not found them convincing. The heterologous protein of the prior art is also an antigen, amending the claims to recite the word "antigen" does not define novelty or an inventive step over the applied prior art.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report.

- [ ] all parts.
- [X] the parts relating to claims Nos. 1-9.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
États-Unis d'Amérique

in its capacity as elected Office

Date of mailing (day/month/year) 07 October 1998 (07.10.98)	
International application No. PCT/US98/01780	**Applicant's or agent's file reference** D5979PCT
International filing date (day/month/year) 30 January 1998 (30.01.98)	**Priority date** (day/month/year) 31 January 1997 (31.01.97)
Applicant BLASER, Martin, J. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

31 August 1998 (31.08.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Marie-Christine Guillemot
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

using *sap*A-specific 3' region forward (*sap*A) and *km* reverse (*km*) primers (left 4 lanes), *sap*A1-specific 3' region forward (*sap*A1) and *km* primers (middle 4 lanes), or *sap*A and *sap*A1-specific 3' region reverse (*sap*A1) primers (right 4 lanes). Figure 2D shows the cumulative restriction maps of the 4 strains presented in Figure 2A-2C. The location of the probes as indicated from the hybridications is shown under the map for each strain. *sap*A x represents an uncharacterized S-layer protein gene cassette; arrows represent direction of transcription; solid lines represent expressed genes, dashed lines represent silent genes; P over bent arrow represents the *sap*A promoter; the heavy line represents the 6.2 kb invertible promoter-containing element, flanked by opposing S-layer protein gene cassettes. The asterisks represent the palindromic putative recombinase recognition sites (TTAAGGAaTCCTTAA) present in the 5' conserved region of each S-layer protein gene cassette (7), and restriction sites are indicated: H, *Hin*cII; N, *Nde*I; P, *Pst*I.

Figure 3 shows the proposed model of molecular events involved in S-layer protein gene cassette rearrangement by DNA inversion. DNA inversion between two oppositely oriented cassettes follows DNA strand exchange at the putative recombinase target site (asterisk) found upstream of each S-layer protein gene cassette within the 5' conserved region (small grey box) (8). Patterned boxes represent variable regions of S-layer proteins gene cassettes. A 6.2kb intervening segment is topologically reversed leading to ordered rearrangement of the S-layer protein gene cassettes. Inversion of DNA segments containing the promoter ('P' over bent arrow) permits expression of alternate S-layer protein gene cassettes (mRNA, arrow). Illustrated are inversion of the 6.2 kb promoter-containing element alone (left), the 6.2 kb element and one (middle) or two (right) S-layer protein gene cassette ORFs and the resultant genotypes. Each of these genotypes has been observed (Figure 2D).

Figure 4 shows a schematic representation of the sapA invertible region, showing the sapCDEF genes, the locations of the divergent sapA and putative sapCDEF promoters (bent arrows), and the clones (pIR15, pIR13, pIR12, and pIR20) from

Replaced by Article

WHAT IS CLAIMED IS:

1. A mutant *C. fetus* strain useful for vaccinating an animal to *Campylobacter fetus*, wherein said strain is mutated to contain a DNA cassette encoding a heterologous protein.

2. The mutant *C. fetus* strain of claim 1, wherein a *sapA* homolog is altered.

3. The mutant *C. fetus* strain of claim 1, wherein said heterologous protein is a S-layer protein.

4. The mutant *C. fetus* strain of claim 1, wherein the encoded S-layer protein represents a chimera between the native S-layer protein and the peptide encoded by the cassette.

5. The mutant *C. fetus* strain of claim 1, wherein said cassette is selected from the group consisting of *Salmonella*, *Shigella*, *Campylobacter jejuni*, *E. coli* 0157:H7, human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV) and animal pathogens.

6. The mutant *C. fetus* strain of claim 1, wherein said cassette contains a 5' binding region and 3' secretion signal region and wherein said protein is inserted between said binding region and said signal region.

7. The mutant *C. fetus* strain of claim 1, wherein said cassette contains a 3' secretion signal but has no binding region.

8. The mutant *C. fetus* strain of claim 1, wherein said protein is selected from the group consisting of an antigen and a therapeutic agent.

9. A method of immunizing a host to develop mucosal and systemic immune responses to an immunogen, comprising the step of administering to said host a pharmacologically effective dose of the strain of claim 1.